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A SALINE MEDIUM FOR MAINTAINING ISOLATED HEART OF *PILA GLOBOSA*, SWAINSON

By M. B. Lal, F.A.Sc. and R. A. Agarwal

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Received October 21, 1967

Abstract

Some known standard salines for molluscs were found unsuitable for maintaining the heart of *Pila in vitro*. A new saline for *Pila* has been devised keeping in view the ionic composition, pH and osmotic pressure of blood of *Pila*. A saline having the composition of blood was not found suitable for maintaining isolated heart of *Pila* but reduction of percentage of potassium made it so. The osmotic pressure was adjusted with addition of glucose which also provides energy. Changes of pH between 5 and 8·5 did not have an effect on the heart-beat of *Pila*. It was suggested that a saline containing only the principal ions of blood was not suitable because other trace and organic substances present in the blood were perhaps playing an important role in the regular beating of the heart. Adjustment in the proportion of different ions and addition of glucose did partly compensate for the absence of these substances.

A suitable saline is always necessary for carrying out experiments on isolated organs. In experiments with marine animals sea-water serves as a very good perfusion medium and isolated organs of marine animals can be kept for very long periods in sea-water. Pilgrim (1953) and Fange (1955) have used diluted sea-water as a perfusion medium for a freshwater lamellibranch (*Anodonta*) but for work on isolated organs of terrestrial and freshwater animals it becomes necessary to find a suitable saline before performing various experiments. The present paper gives an account of processing a suitable saline for maintaining the isolated heart of *Pila in vitro* for experimental purpose.

The details of the technique followed are given here.

The pericardium was carefully slit open so that the heart remained undamaged. A piece of surgical ligature silk of '0' thickness was tied around the visceral aorta, a little away from the bifurcation of the aortic trunk. The cephalic aortal end was left open for the perfusion fluid to pass. Care was taken to include some of the surrounding mantle wall while

tying the thread so that the silk thread may not cut through the thin-walled visceral aorta.

A second silk thread was now passed from below the auricle in its middle and a small transverse incision was made in the auricle a little behind the thread. A fine glass canula attached to the perfusion assembly was now passed into the auricle through the incision and the thread was securely tied around it.

The heart was now detached from the body of *Pila* by cutting through the cephalic and visceral aortae and the auricle a little behind the point of the insertion of the canula. The aortic end of the heart was tied to a writing lever in order to record the heart-beats. The heart along with the assembly was now put in a bath filled with saline and the heart-beat was studied during and after perfusion (Fig. 1).

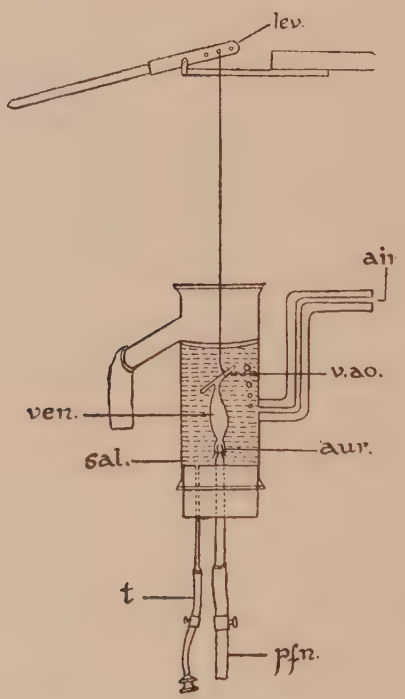

FIG. 1. Diagram of the bath used for perfusing the heart of *Pila globosa*.

air., inlet for aeration; *aur.*, auricle; *lev.* lever; *pfn.*, tube for perfusion; *sal.*, saline; *t.*, tube for experimental fluid and for washing; *v.ao.*, visceral aorta; *ven.*, ventricle.

The experiment was carried out in a temperature-controlled laboratory with temperature range of 16°–22° C. The aeration of the bath was,

obtained by blowing fine bubbles of air from a side tube connected to an aspirator pump of the type commonly used in small fish aquarium. As the bubbles are small and are blown at a low rate they do not disturb the heart mechanically. At the bottom of the bath there is a tube in the perfusion assembly for drawing out the bath fluid and introducing experimental fluids. A slow constant drip of the perfusion fluid under constant pressure was maintained (Fig. 1).

In recording the heart-beat a muscle lever which was 13 cm. long and was counter-weighted to provide a tension of approximately 500 mgm. was used to write on a smoked Kymograph drum moving with a speed of about 1·2–1·5 cm./minute.

The following perfusion media were tried:

1. *Hedon fleig saline.*—This saline, which has a high pH and low potassium and calcium content, can retain the activity of the heart for only about 10 minutes, after which the heart stops (Fig. 2).

2. *Jullien's solution used for Helix.*—This solution (having a composition NaCl—6·5 gm.; KCl—0·14 gm.; $CaCl_2$—0·12 gm.; $NaHCO_3$—0·20 gm. dissolved in one litre of distilled water) does not maintain the isolated heart of *Pila* for more than 3–4 hours during which period also the beats are very irregular. The heart ultimately stops in diastole. A modification of the above solution by increasing its calcium content does not help much in maintaining the heart although the beats become more regular.

3. *Divaris and Krijgsman's solution used for Cochlitoma.*—This solution (having a composition NaCl—5·10 gm.; KCl—0·15 gm.; $CaCl_2$—0·75 gm.; $NaHCO_3$—0·20 gm.; $MgCl_2$—0·50 gm.; Glucose—1·00 gm. dissolved in one litre of distilled water) does not maintain the heart of *Pila* for more than an hour. Apparently these salines did not prove satisfactory because of the unsuitable Ionic composition, Osmotic pressure, and pH value of the fluid.

As the ionic composition of the saline is of foremost importance it is necessary that not only the correct quantity but the correct proportion of the different ions is used. Saxena (1957) gave the concentration of the different inorganic ions in mg./100 ml. of blood of *Pila*, but the preparation of a saline by suitable combination of these salts in which the total quantity of anions was lesser than the cations as given by Saxena (1957) was met with difficulties. Since for the proper functioning of the heart of molluscs the quantity and proportion of the cations like Na, K, Ca and Mg are more

important than the anions, an attempt was made to keep the quantities of the basic ions constant and adjust the amount of anions accordingly. This was also necessitated from the fact that a combination of the salts, NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2HPO_4, $MgSO_4$ could not provide a saline in which the cations and anions were exactly in the same proportion as given by Saxena (1957). By a suitable adjustment, a modified saline was prepared (Composition: NaCl—3·168 gm.; KCl—0·373 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·254 gm.; $MgSO_4.7H_2O$—0·028 gm.; $Na_2HPO_4.12H_2O$—0·063 gm. dissolved in one litre of distilled water).

In the above solution the amount of Na^+, K^+, Ca^{++}, Mg^{++}, SO_4^{--} PO_4^{--} and Cl^- ions dissolved in 100 ml. of distilled water would correspond to Na—125·7 mg.; K—19·3 mg.; Ca—30·7 mg.; Mg—3·3 mg.; PO_4—1·7 mg.; SO_4—1·1 mg.; Cl—284·18 mg.

Moreover the quantities of the Na^+, K^+, Ca^{++}, Mg^{++}, SO_4^- and PO_4^{--} ions in this saline are the same as those found in the blood of *Pila*. Only the quantity of Cl^- is higher. This solution shows a pH range of 4·5–5·5. In this modified *Pila* saline the heart of *Pila* survives longer upto 5–6 hours but the heart-beats do not remain regular (Fig. 3).

Attempts were made to vary the concentration of various ions (K, Ca, Mg and Na), and observe the effect on the regularity of the heart-beat. It was noticed that the absence of potassium from the saline causes an increase in amplitude and frequency of the heart-beats which ultimately become irregular and then the heart stops in diastole in about 50 minutes.

To such a solution (Composition; NaCl—3·197 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm.) completely lacking in potassium, varying quantities of potassium salt KCl were added. For this purpose a stock N/100 KCl solution was prepared and measured quantities of this were added to the above solution. It was found that only a little addition of KCl (2 c.c. N/100 ml. in 100 ml. of saline) greatly increases the survival period of the heart which could live for more than 24 hours. With the addition of different concentrations of potassium in this way, it was found that with 10 c.c. N/100 KCl in 100 ml. of the above saline the heart would beat regularly and remain alive for more than 36 hours. A solution was, therefore, prepared containing NaCl—3·197 gm.; KCl—0·074 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm. dissolved in one litre of distilled water.

Besides the ionic composition of the saline, its osmotic pressure and pH value are equally important.

It was, therefore, decided to balance the osmotic pressure and pH value of the above saline according to those present in the blood of *Pila*. The osmotic pressure of the above saline was found lower than that of the blood of *Pila*. Moreover, it did not contain any organic nutritive material to provide energy to the isolated heart under experiment. It was, therefore, thought desirable to make the above saline isotonic with blood of *Pila* by the addition of glucose, and adjustment of the osmotic pressure was done by comparing the depression of freezing point of the blood of *Pila*. The average lowering of the freezing point of blood was found to be 0·29° C.

The saline was accordingly adjusted by adding 1 gm. of glucose per 1,000 c.c. of saline. This saline now becomes nearly isotonic with the blood of *Pila*, as the freezing point in this case is also 0·29° C. The addition of glucose would provide energy to the heart during experimental *in vitro* condition for long periods. This saline after addition of glucose shows a pH value of 5–5·5. Since the normal blood of *Pila* is slightly alkaline small quantities of $NaHCO_3$ were added to this solution to bring the pH to near the normal condition although it was found that changes within the range 5–8·5 do not materially affect the activity of the heart of *Pila*. Addition of 0·25 gm. of $NaHCO_3$ to one litre of the saline solution makes its pH 7·5. It has been seen that the addition of extra Na^+ and CO_3^{--} ions does not affect the working of the heart. Thus the saline of the following composition was found to be the most suitable for the heart of *Pila* as was clear from the subsequent experiments: NaCl—3·197 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm.; KCl—0·074 gm.; Glucose—1·00 gm.; $NaHCO_3$—0·25 gm. disssolved in one litre of distilled water.

In the above saline it was possible to maintain the isolated heart of *Pila* living in almost normal condition for 48–72 hours (Fig. 4).

The preparation of physiological (balanced) salines should aim at maintaining *in vitro* isolated parts of experimental animals in as nearly a normal living state as possible. Inside the body of the animal the tissues are bathed in blood or haemolymph, yet as has been mentioned earlier, a saline resembling the inorganic composition of the blood of *Pila* was not found a satisfactory substitute for blood. In several cases fluids having the inorganic composition of blood have not been found suitable as physiological salines, although Jullien *et al.* (1955) have used the same ionic concentration of calcium, potassium and sodium as in the blood of Helicides for preparing saline,

The role of different ions is still far from clear as in spite of the fact that blood is composed of a large number of inorganic substances, Zoond and Slome (1928) have used a simple solution of sodium chloride and calcium chloride as a physiological saline for *Palinurus* and *Octopus*. Hogben (1925) has used the same two salts for *Mya* and Otis (1942) for *Ostrea*.

The sea-water which closely resembles the composition of body fluids of marine animals also acts as an excellent saline for most of them. Harvey (1957) has pointed out that sea-water contains ten principal substances, which are Sr, Br, H_3BO_3, SO_4, and Na, K, Ca, Mg, Cl and CO_3 ions but out of these only six (*viz.*, Na, K, Ca, Mg, Cl and CO_3 ions) are generally used in physiological salines. Besides these a number of minor inorganic constituents are present in the sea; there are several organic substances in solution and a number of organic and inorganic particles in suspension.

All these substances make the sea-water, so that when sea-water is used for perfusion there are a large number of substances both organic and inorganic contributing towards the healthy existence of the heart. It is likely, therefore, that the substances present in traces in the sea-water exert some definite healthy role on cardiac metabolism, similar to the effect exerted by trace substances present in the blood. It is possible that by altering the proportion of the major ions of the blood the absence of the trace and organic substances is partly compensated.

The hydrogen ion concentraton also plays an important role in the metabolism. It has, however, been noticed by us and other workers like Meeter (1955), Zoond and Slome (1928), and Otis (1942), that small changes in pH do not affect the working of the heart very much. The studies of Kokubo (1929) have shown that in oysters the pH of blood decreases in summer and increases in winter; further, it also changes with the change of pH in the environmental sea-water. It is possible, therefore, that as the heart is subjected to such changes in pH during normal conditions, it is not sensitive to mild changes of pH.

The present studies reveal that for preparing a suitable saline in case of *Pila*, modifications in the ions as present in the inorganic composition of its blood and addition of glucose are essential. The modification probably compensates for the absence of many trace substances and the organic materials present in the blood.

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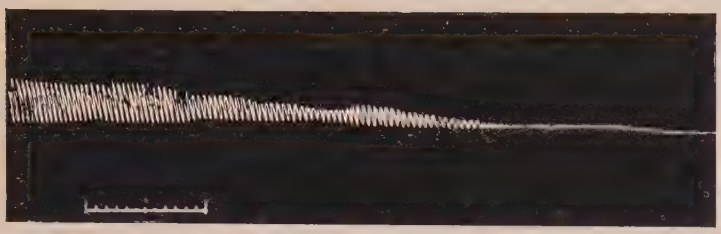

FIG. 2. Graph showing the heart beat of *Pila globosa* in Hedon Fleig's saline. Time scale—2 minutes.

FIG. 3. Graph showing the heart beat of *Pila globosa* in a saline resembling the inorganic composition of its own blood. Time scale—2 minutes.

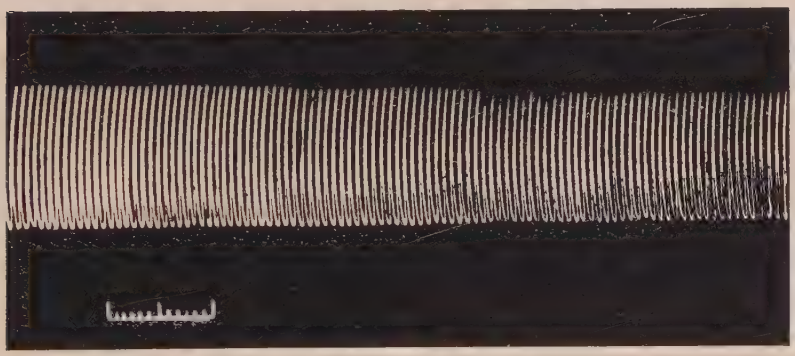

FIG. 4. Graph showing the regular beat of the isolated heart of *Pila globosa* in *Pila* saline. Time scale—2 minutes.

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ANATOMICAL STUDIES IN *CLERODENDRUM SPLENDENS* G. DON

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Received October 25, 1967

(Communicated by Prof. V. Puri, F.A.SC.)

Abstract

The present paper deals with the anatomical studies in the leaf of *Clerodendrum splendens* G. Don. The extra-floral nectaries are patelliform and originate from a single epidermal papillate cell. They secrete sugary fluids. The diffuse type of brachysclereids and the spheroidal crystalliferous sclereids occur in the mesophyll, mid-rib region of the leaf and the ground tissue of the petiole. The vascular bundles in the mid-rib region and the petiole are arranged in a ring. The phloem does not form a continuous arc. The smaller vascular bundles in a ring originate from vascular meristems with delayed differentiation. Accessory bundles in the wings of the petiole seem to originate as a result of fusion of two separate strands. The vascular cambium with its characteristic radial alignment of cells is present in between xylem and phloem in the leaf and the petiole.

Introduction

Foliar nectaries are of widespread occurrence among the angiosperms on various organs of the plant. In the family Verbenaceae they are reported in several lignose species (Metcalfe and Chalk, 1950). However, our knowledge regarding the structure, ontogeny and function is rather meagre. The structure and ontogeny of these organs in the Verbenaceae have been studied by some workers (Maheshwari, 1954; Chavan and Deshmukh, 1959, 1960, 1963, 1964 *a*, 1964 *b*; Maheshwari and Chakrabarty, 1966) in few members. The nectaries occur on the lower surface of the leaf more nearer the mid-rib region (Fig. 1: 1, 2 and 14). The mature nectaries appear as small dark glistening dots to the naked eye.

Sclereids are reported to be present in various organs of the plant (Esau, 1962; Foster, 1949). Foliar sclereids are not reported in the family Verbenaceae (Solereder, 1908; Metcalfe and Chalk, 1950; Rao, 1951).

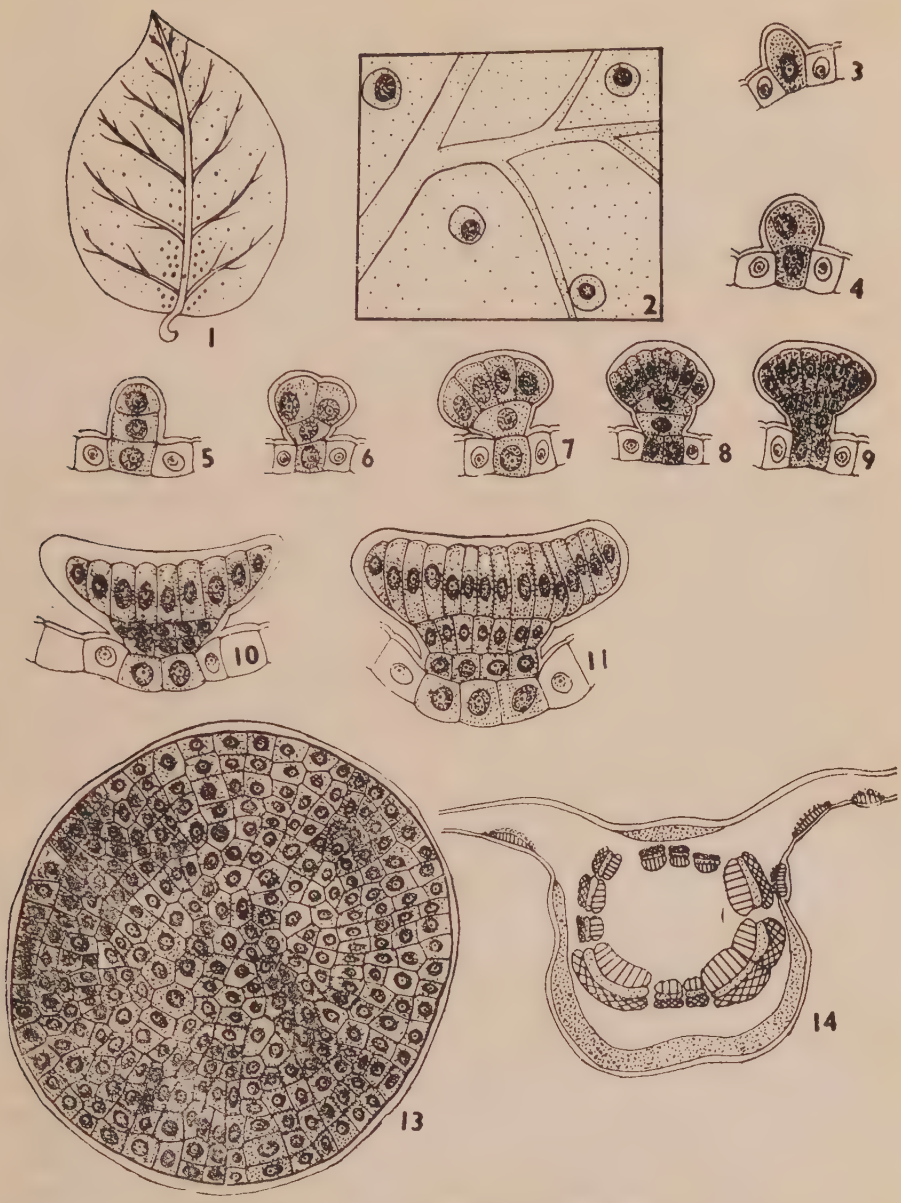

FIG. 1. 1–11 and 13–14. 1. Lower surface of leaf showing location of foliar nectaries in the form of dots, $\times\frac{1}{2}$. 2. Part of the leaf magnified showing nectaries in surface view, ×4. 3. Epidermal nectary initial, ×440. 4. Same after periclinal division showing basal and an outer cell, ×440. 5. 3-celled stage showing a basal, a primary stalk cell (middle) and a body cell, ×440. 6–11. Stages in the development of the foliar nectary, ×440. 13. T.S. passing through the head of a nectary, ×440. 14. Diagrammatic representation showing the position of nectaries in T.S. of leaf, ×27.

Maheshwari (1954) reported macrosclereids in the fruit pericarp of *Lippia nodiflora* Rich. a member of Verbenaceae.

The vascular cambium appears in strips in most petioles and leaf veins that show secondary growth (Esau, 1965). But very little information is available on the detailed development and activity of cambium *in situ* in the petiole.

The structure, ontogeny and functions of foliar nectaries, distribution and development of foliar sclereids and activity of vascular cambium in the leaf of *Clerodendrum splendens* G. Don form the subject of the present study.

MATERIALS AND METHODS

The material of *Clerodendrum splendens* G. Don was collected from local gardens where it is cultivated usually as a beautiful climber. The shoot tips, the young and mature leaves were fixed in FAA and the customary methods of dehydration, infiltration and embedding were followed. The sections were cut at the thickness of 8 microns, and stained with Regaud's haematoxylin and fast green. The hand sections of the old petioles and leaves were also taken. Temporary whole mounts of leaves and petioles cleared in 2·5% warm NaOH were also observed for sclereids. Leaf pieces were boiled in a mixture of equal proportion of Fehling's solution A and B and the peals of the lower epidermis were observed for the presence of sugars in the secretory tissues of the foliar nectaries.

OBSERVATIONS

I. Foliar Nectaries

Development:—The papillate nectary initial is differentiated on the lower epidermis of the leaf. It has a prominent nucleus and a dense cytoplasm (Fig. 1 : 3). As in *Duranta plumieri* Jacq. it undergoes periclinal division to form an outer and a basal cell (Fig. 1 : 4). The outer cell divides again periclinally to form a primary stalk cell and a body cell or the terminal cell (Fig. 1 : 5). The primary stalk cell divides firstly periclinally and then anticlinally to form a 2-layered short but broad stalk (Fig. 1:8 and Fig. 2:12). The initial divisions in the body cell are anticlinal but the later divisions are irregular (Fig. 1 : 6–11 and Fig. 2 : 12). This histogenic activity results in a broad saucer-shaped head or shield (Fig. 2 ; 12 and Fig. 1 : 13). The basal cell also divides simultaneously to keep pace with increased breadth of the stalk region (Fig. 1 : 6–11 and Fig. 2 : 12). They remain in continuation with the epidermis

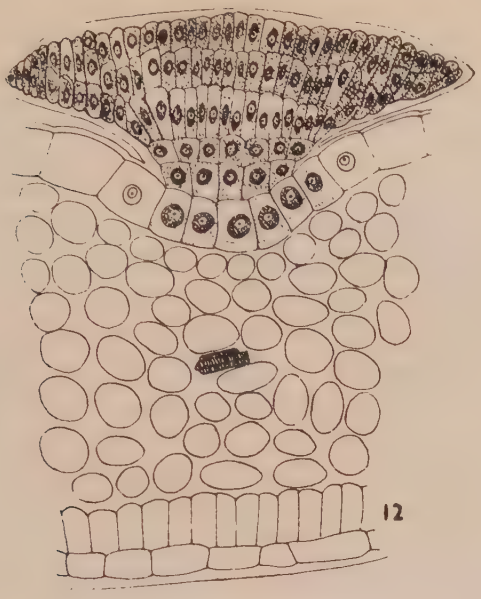

FIG. 2. 12. Mature nectary showing a base, a stalk and the main body, the secretory tissue, ×440.

but differ from epidermal cells in having larger nuclei and rather denser cytoplasm. The general development is thus similar to that of *Duranta plumieri* Jacq. (Maheshwari, 1954).

Structure and function.—The mature nectaries are patelliform and situated in shallow depressions. The main body of the nectary consists of 1–3-layered, long, narrow, palisade-like epithelial cells with dark staining properties (Fig. 2: 12). There is a thin layer of cuticle running over the nectary in continuation with the general surface of the leaf. The stalk is 2-layered consisting of rectangular cells with their anticlinal walls rather thickened (Fig. 2: 12). The cells of the basal region are similar to the stalk cells except that they are larger in size. Applying the concept of Zimmermann (1932) and Maheshwari (1954) the nectaries are either of depressed or surface type. In cross-section of the body region of the nectary consists of small parenchymatous cells with minute nuclei (Fig. 1: 13).

According to Chakravarty (1937, 1948) and Maheshwari (1954) the extra-floral nectaries are said to allure protective ants against pests and excrete superfluous fluids from the plant body. In *Clerodendrum splendens* G. Don the secretion appears as a transparent drop by which the small insects are attracted. The secretory activity is more during the cloudy

days and winter. The micro-chemical test with Fehling's solution A & B shows the presence of reducing sugars in the secretory tissue of the nectary. Occasionally starch grains were also observed.

II. Foliar Sclereids

Distribution of sclereids.—The leaf is bifacial with 1–2-layered palisade parenchyma. The spongy parenchyma is well developed with moderate intercellular spaces. The brachysclereids are found scattered in the spongy parenchyma of the leaf in groups of 2–3. The sclereids have a thick stratified wall with narrow to large lumen and narrow pit-cavities. The pit-canals are either simple or ramiform having straight or oblique disposition. In the mid-rib region and the petiole the sclereids are distributed in the ground tissue. In this region the sclereids occur in groups of 3–20 or more forming distinct strands, rarely salitary. Star-shaped, rod-like, rectangular and square crystals of Ca-oxalate are either deposited in the lumen or embedded in the wall of the sclereids. Rao (1957) designated such sclereids as crystalliferous.

Ontogeny.—In the embryonic condition the leaf has little differentiation into palisade and spongy cells. Then the mesophyll differentiates into palisade and spongy parenchyma. Some of the spongy parenchyma cells differentiate as sclereid initials (Fig. 3: 1). The sclereid initials sometimes occur in groups of 2–3. They have a prominent nucleus and a dense cytoplasm. In the further development the cytoplasm appears in strands and the vacuoles appear either from the periphery or the centre (Fig. 3: 1). The cells begin to show sclerosis (Fig. 3: 2). The secondary wall shows stratifications indicative of gradual centripetal deposition of cell wall material. Ultimately the nucleus degenerates and the cytoplasm persists in the lumen for a long time (Fig. 3: 2). The secondary wall shows straight or oblique. pit-canals closely situated or spread over without any uniformity (Fig. 3: 3, 4 and 5).

III. Vascular Cambium in Leaf

The single-layered cutinised epidermis is followed by 4–5 layers collenchymatous hypodermis (Fig. 4: 3). The parenchymatous ground tissue shows diffusely arranged aggregates of crystalliferous spheroidal sclereids (Fig. 4:3). The ground tissue also contains needle-like, rectangularly star-shaped, rod-like and square crystals of Ca-oxalate and also contain starch grains. 12–13 vascular bundles of varying sizes are arranged in a ring (Fig. 4: 3). The smaller vascular bundles appear to originate from

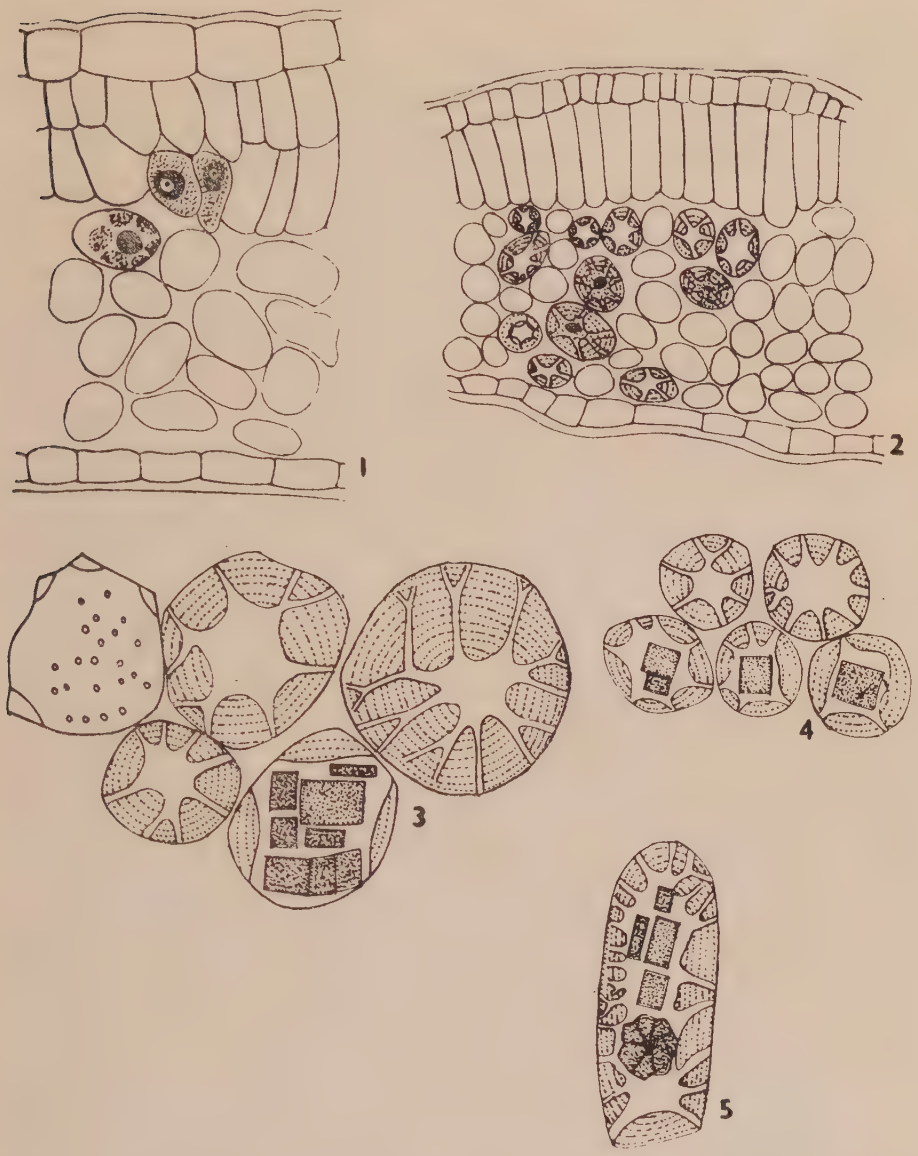

FIG. 3. *Foliar Sclereids* (1–5, ×440). 1. T.S. of young mature leaf showing sclereid initials. 2. T.S. of mature leaf showing brachysclereids nearer the margin (note degenerating nuclei and cytoplasm in some). 3. Sclereids from petiole region (note crystals in lumen). 4. Sclereids from mid-rib region (note crystals in lumen). 5. Sclereid in L.S. view (note crystals in lumen).

vascular meristems whose differentiation is delayed. The number of accessory vascular bundles in each wing are 2 but they may also fuse to form a single bundle (Fig. 4: 3). The characteristic vascular cambium is present

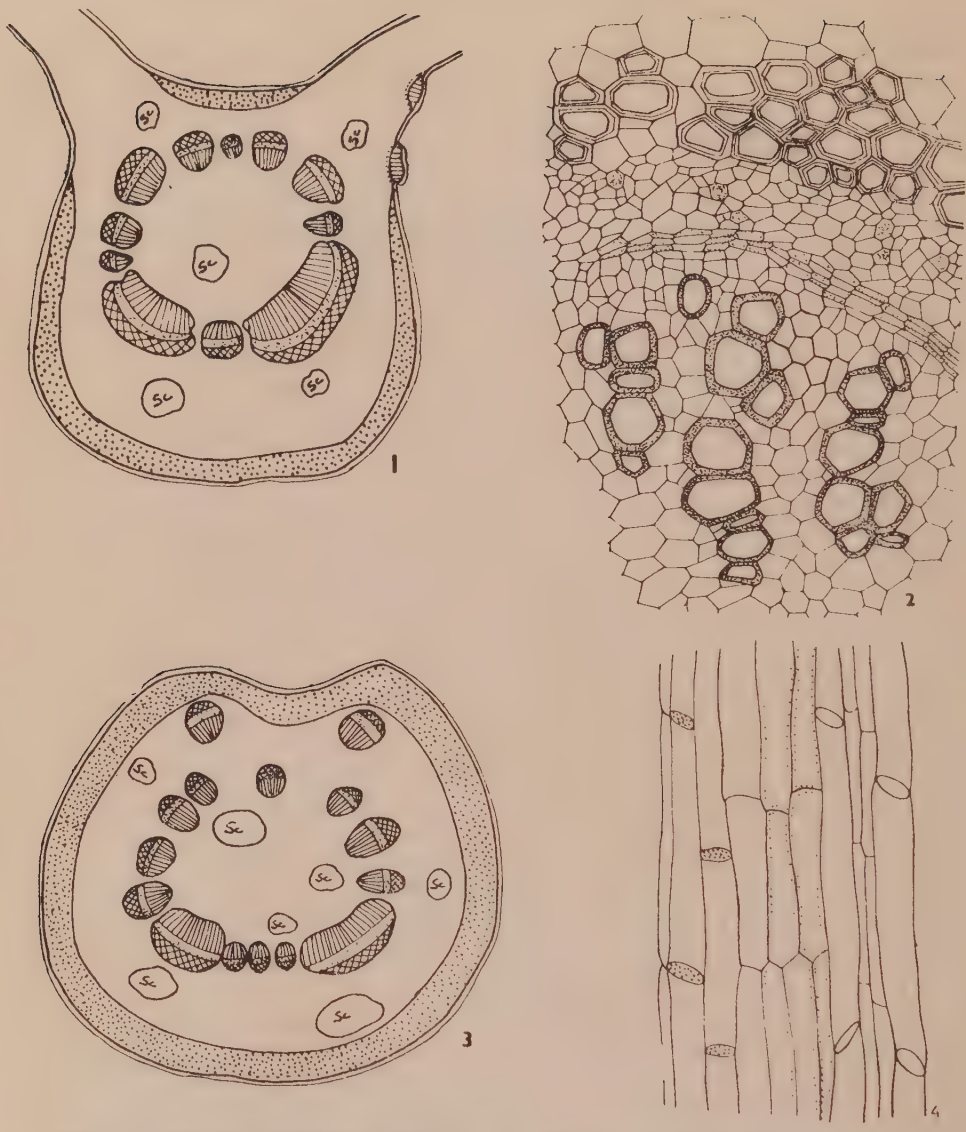

FIG. 4. *Vascular Cambium in Leaf* (1–4). 1. T.S. of leaf, diagrammatic, ×110. 2. T.S. of leaf showing vascular cambium in between xylem and phloem, ×440. 3. T.S. of petiole, diagrammatic, ×110. 4. L.S. of petiole showing fusiform cambium cells, ×440.

between xylem and phloem. The phloem does not appear in continuous arc as reported by Metcalfe and Chalk (1950), whereas the xylem is in the form of discrete bundles (Fig. 4: 3). The mid-rib region of the lamina also shows limited cambial activity (Fig. 4: 2). About 11 vascular bundles are observed in a ring, of varying sizes, in the mid-rib region of the leaf

(Fig. 4: 1). As in the petiole, the smaller vascular bundles show delayed differentiation. Sclerenchymatous bundle caps which are probably primary phloem fibres are present in all the bundles of the leaf and the petiole (Fig. 4: 1, 3). The cambium with its characteristic radial alignment of cells is observed (Fig. 4: 2). In longisection the fusiform initials of cambium are distinct (Fig. 4: 4).

Discussion of Conclusions

The structure, development and functions of foliar nectaries, distribution and ontogeny of foliar sclereids and the activity of vascular cambium in the leaf of *Clerodendrum splendens* G. Don are described here.

The foliar nectaries are epidermal in origin and situated in shallow depressions on the lower surface of the leaf. At maturity they are patelliform with 1-layered base, 2-layered stalk and a body consisting of 1–3-layered palisade-like epithelial cells. Test with Fehling's solution showed the presence of reducing sugars in the secretory tissue. The secretory activity is more during cloudy days and winter.

The sclereids exhibit a diffuse pattern of distribution. The foliar sclereids are transformed spongy cells while those in the mid-rib region are transformed parenchyma cells. Very rarely they are transformed palisade cells. Brachysclereids occur in the laminar region while the crystalliferous spheroidal sclereids occur in the ground tissue of the mid-rib region and the petiole.

According to Metcalfe and Chalk (1950), the transection of the petiole of *Clerodendrum* shows vascular tissue in the crescent form with incurved ends. The xylem appears in discrete group and the phloem in continuous arc. In the wings accessory vascular bundles are also present. Samantrai and Kabi (1953, 1954*a*) and Mitra and Bose (1957) described secondary thickening in the leaves of the members of Nyctaginaceae, Amaranthaceae and Chenopodiaceae after giving some chemical treatment. The present observations on the leaf of *Clerodendrum splendens* G. Don are different from those given by Metcalfe and Chalk (1950), the secondary thickening in the mid-rib region of the leaf and the petiole is studied without giving any type of chemical treatment. In the petiole and the mid-rib region of the leaf the vascular bundles are arranged in a ring. The phloem does not form a continuous arc. The smaller vascular bundles originate from vascular meristem with delayed differentiation. A pair of accessory vascular bundles in the wings of the petiole appear to originate as a result of fusion of two

separate strands in each wing. Sclerenchymatous bundle caps which are primary phloem fibres are present in all the vascular bundles. The vascular bundles are of varying sizes. The vascular cambium with its characteristic radial alignment of cells is present between xylem and phloem.

ACKNOWLEDGEMENTS

The author wishes to record his deep sense of gratitude to Prof. V. Puri and Prof. A. S. Foster for reviewing this paper. Thanks are also due to Dr. J. J. Shah for reading the manuscript and giving useful suggestions. I thank Mr. Poulos and Mr. M. A. Francis for helping me in photography.

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PRESENCE OF A GROWTH INHIBITOR IN THE TUBERS OF NUTGRASS (*CYPERUS ROTUNDUS* L.)

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(Communicated by Dr. L. D. Kapoor, F.A.SC.)

Nutgrass (*Cyperus rotundus* L.), a perennial weed of Cyperaceae, is of wide occurrence throughout the temperate and tropical regions. This weed possesses a high potentiality of propagation by tubers (Baker, 1964). Aerial growth of this weed, observed under favourable conditions of moisture and temperature, is not as important as the underground structures that generally weigh 4 to 5 times more than the foliage (Hauser, 1962). This weed seldom attains a height of more than one-and-a-half feet or so, but the decline in the productivity and vigour of crops, infested with nutgrass, is so severe that it can never be overlooked under field conditions. These deleterious effects may not be solely attributed to the rob of available moisture and nutrients, and competition for light and space by such a short statured plant that produces not more than about 2 tons of aerial vegetation (Baker, 1964) rather to the antagonistic effects of exudates from its sub-aerial parts which keep the growth and vigour of competing vegetation suppressed.

As early as 1882, de Candole pointed out the specific inhibition of flax by spurge (*Euphorbia* sp.) and of oats by thistle (*Cnicus* sp.), and postulated the production of specific toxic substances by these weeds. Evenari (1949) and Bentley (1958) have summarized the work on naturally-occurring inhibitors. Numerous reports attest to the deleterious effects of quack-grass (*Agropyron repens* L.) on other plant species (Kommedahl, *et al.*, 1959; Kacarava, 1961; Welbank, 1959). The roots and other underground organs of quack-grass (Le Tourneau *et al.*, 1957; Ohman *et al.*, 1964; Osvald, 1948), *Cnicus arvensis* (Kacarava, 1961), *Setaria glauca* (Yakum *et al.*, 1961), and several others (Lawrence and Kilcher, 1962) contain phytotoxic substances. Plants liberating such substances, either as exudate and/or leachate of decomposing dead organs, become victors in the struggle for existence in the plant community primarily by inhibiting the germination and growth of susceptible species.

Eventually, in September 1966, the aqueous extract of nutgrass tubers, involved in an experiment on the antagonism and synergism of auxin herbicides, drew the attention as it inhibited the seed germanation and growth of some crop seedlings. This observation led to some systematic work to demonstrate the presence of some water-scluble substance(s) inhibitory for germination in nutgrass tubers, the crop species affected by it, and the degree of such inhibition.

Materials and Methods

Nutgrass tubers were collected from the fallow lands of Punjab Agricultural University, Hissar Farm. Water extract of tubers was obtained by soaking 50 gm. of slightly crushed tubers in a litre of distilled water for a period of 50 hours at 30° C. The filtrate, obtained from it, was taken as the water extract. Seeds of 10 crops, *viz.*, Bajra (*Pennisetum typhoides*), var. S. 530; cowpeas, No. 1; sorghum (*Sorghum vulgare*), var. J.S. 163; maize (*Zea mays*), Hybrid Ganga 101; black gram (*Phaseolus mungo*), var. 1–1; cotton, H–14, and local variety of paddy, sesame, sunnhemp and groundnut were tested. Only rainy season crops were included in this experiment, as nutgrass fluorishes well in this season. This experiment consisted of 20 treatments, each replicated 4 times. The experiment was repeated twice, first in sand and the other on filter-paper. Fifteen seeds were placed, equally apart, in 6 in. glass Petri dishes. Seeds were covered with 400 gm. of well-washed coarse sand, later moistened with either 50 ml. of distilled water or tuber extract. Petri dishes were held at $30 \pm 2°$ C. Number of seedlings emerged out of sand, and their length were recorded for 8 subsequent days. In the other set, planned to observe the characteristic inhibition, seeds were put over single circles of Whatman filter-paper No. 1, 150 mm. dia., in place of sand. The volume of water or tuber extract, added to each Petri dish, was reduced to 5 ml. for proper and sufficient wetting of filter-papers. Petri dishes were kept in the germination chamber, held at $30 \pm 2°$ C. On subsequent days, the number of germinated seeds were recorded and the characteristic inhibitory effects were marked.

Results and Discussion

Table I represents the data on the percentage of seedlings emerged from the sand medium. It is obvious that the aqueous extract of nutgrass tubers inhibited the emergence of crop seedlings. Except maize, all crop species were affected to varying degrees. The length (Text-Fig. 1) and vigour of seedlings, in general, observed from the very beginning, were greatly

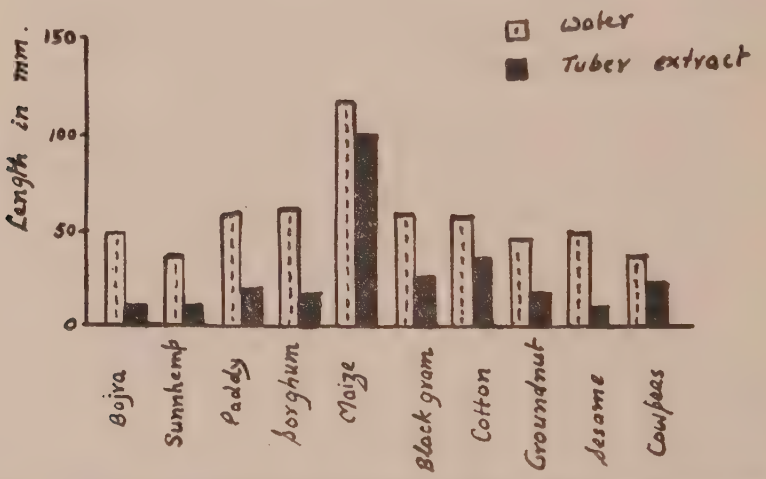
TABLE I

Per cent seedling emergence of crops with and without nutgrass tuber extract ; as recorded on 25–8–1967 (8 days after sowing)

Treatment	Bajra	Sunnhemp	Paddy	Sorghum	Maize	Black-gram	Cotton	G. nut	Sesame	Cow peas	A
Water ..	86·6	100	76·6	43·3	85·0	96·6	88·3	56·6	55·0	93·3	
Tuber extract	23·3	45·0	60·0	40·0	85·0	76·6	65·0	20·0	10·0	40·0	
Average ..	55·0	72·5	68·3	41·6	85·0	86·6	76·6	38·3	32·5	66·6	

C.D. at 5% probability = 8·17% (for seed treatment)

hampered by the tuber extract. Radicals emerging from groundnut seeds, which received tuber extract, were observed coming upwards, as against their usual downward movement (Plate II, Fig. 1).



TEXT-FIG. 1. Effect of nutgrass tuber extract on the length of crop seedlings, 8 days after sowing.

When filter-paper was used as the germination medium, percentage of seed germination (Table II) was less with tuber extract as compared to controls. Maize and cotton resisted the toxic effects in germination; less adverse effects were observed in case of sunnhemp and black-gram, while all the rest crops, so far as their germination is concerned, were severely affected.

From these two experiments, the presence of some phytotoxic substance(s) in nutgrass tubers is evident. Seedling emergence, an ultimate

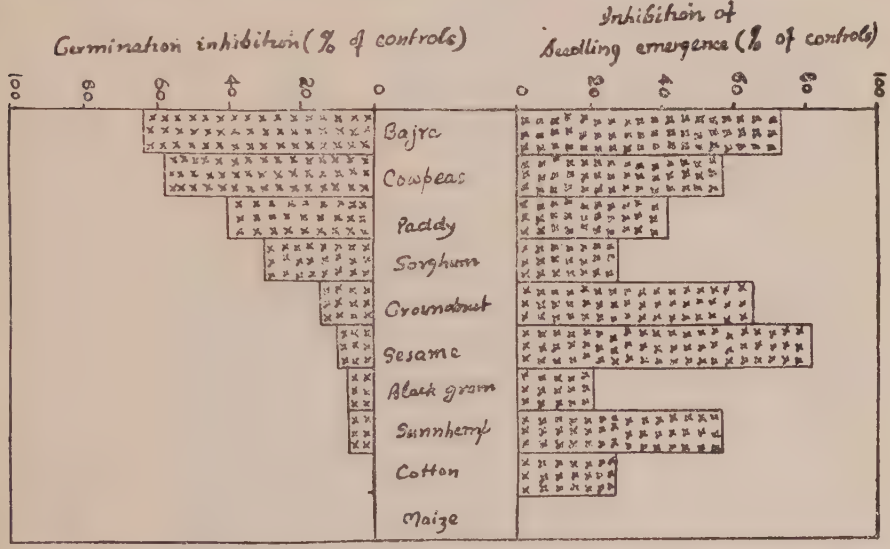
TABLE II

Germination percentage of different crop seeds treated with nutgrass tuber extract and water; as recorded on 31–8–1967 (*7 days after sowing*)

ment	Bajra	Sunnhemp	Paddy	Sorghum	Maize	Black gram	Cotton	G. nut	Sesame	Cow peas	Average
..	100	100	73·3	80·0	100	100	70·0	83·3	95·0	91·6	89·3
xtract	35·0	93·3	41·6	55·0	100	93·3	70·0	70·0	85·0	40·0	68·3
..	67·5	96·6	57·5	67·5	100	96·0	70·0	76·6	90·0	65·8	..

C.D. at 5% probability = 7·66% (for seed treatment)

result of germination and early growth of seedlings, is reduced by tuber extract. Text-Figure 2 shows that inhibition of germination in cowpeas, paddy and sorghum; inhibition of both germination and post-germination seed-



TEXT-FIG. 2. Per cent. inhibition of germination and seedling emergence of crops by aqueous extract of nutgrass tubers.

lings' growth in Bajra, groundnut, sesame, black-gram and sunnhemp, and only retardation of growth of germinated cotton seedlings contributed towards this large reduction in seedling emergence. Maize was totally indifferent to nutgrass tuber extract during germination and early stages of growth but its height and vigour were always brought down in later stages (Plate II, Fig. 2). The inhibitory effects of nutgrass tuber extract on the shoot height

and vigour of almost all crops were quite identical to the root extracts of quackgrass (Kommedahl *et al.*, 1959; Osvald, 1948) that was observed with several crop species.

Post-germination inhibition of growth, which was very clear in groundnut and sesame, was primarily confined to radical and root hairs that ultimately govern the growth of aerial shoot. In sesame (Plate II, Fig. 3) root hairs completely degenerate by the tuber extract. Upward movement of radical in groundnut might result due to upset of internal auxin balance.

CONCLUSION

Tuber extract of nutgrass, when applied to the growing medium, inhibited the germination and growth of 10 crop species under the laboratory conditions. On the basis of above findings, the presence of some phytotoxic substance(s), however, in nutgrass tubers has been confirmed. Although the germination and growth of all the crops, included in this experiment, was inhibited by the phytotoxins present in nutgrass tubers yet the degree of such inhibition varied with different crop species.

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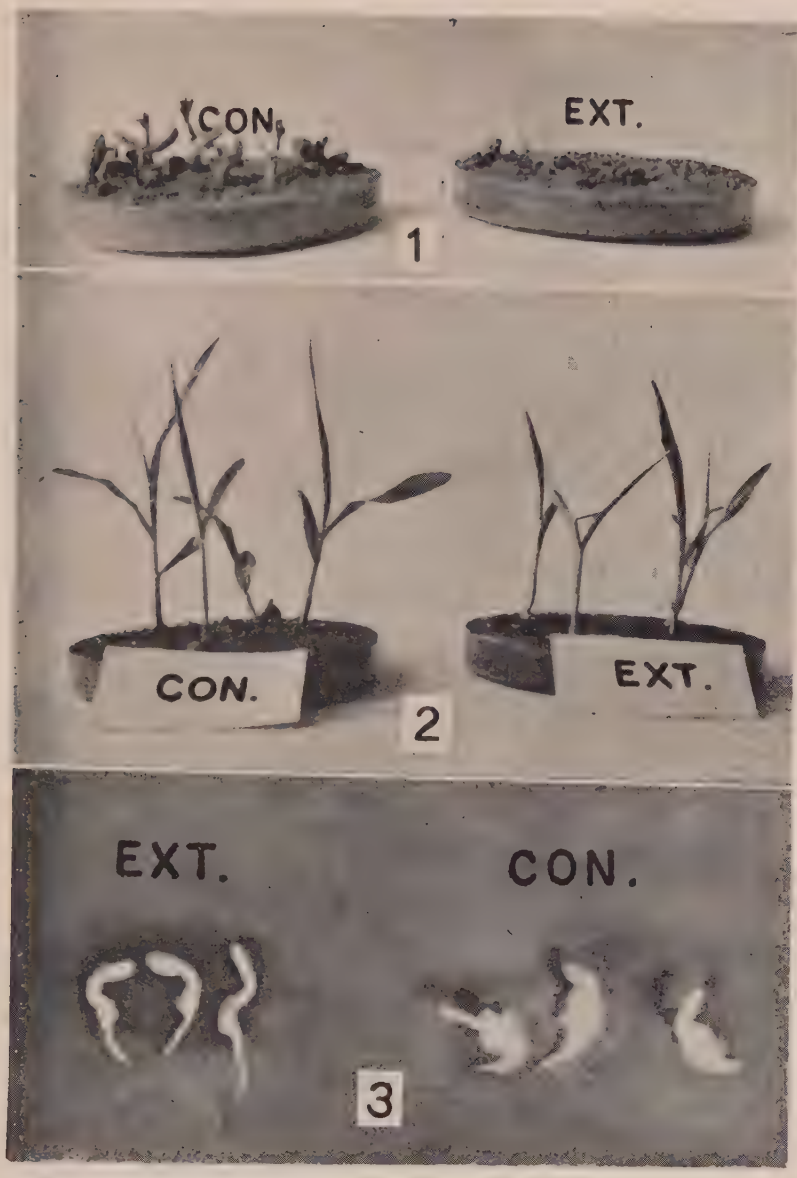

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ON SOME ARCHIANNELIDS FROM THE BEACH SANDS OF WALTAIR COAST

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Abstract

The paper reports the occurrence of twelve species of Archiannelids in the interstitial sands of the Waltair beach. Of these, six European species are reported for the first time from Indian waters and their occurrence on the Indian coast is of great interest from a Zoogeographical aspect of their distribution.

Introduction

While engaged in the study of the interstitial fauna of the beach sands of Waltair coast, several interesting species of Archiannelida were encountered in the collections. The previous records of the fauna in Indian waters are known from the works of Aiyar and Alikunhi (1944) and Alikunhi (1948), who have described seven species from the coasts of Madras, Krusadai Island and Cranganore. The present paper lists altogether twelve species of archiannelids, half of which are recorded for the first time from Indian waters. The occurrence of some of these European species on the Indian coast indicates the extent of their geographical distribution.

The archiannelids were collected by taking fresh sand samples in a glass beaker and vigorously swirling them with sea-water when the worms were shaken off to the surface. The supernatant water was quickly decanted off into a petridish from where the animals were picked up with a pipette under a binocular microscope. The forms were narcotised with weak solutions of Magnesium chloride and fixed in Bouin's fluid. The worms were usually collected in the intertidal sands with coarse texture where the wide interstitial spaces allowed free movements for the worm. The temperature in the habitat varied from 25–30° C. while the salinity ranged from 24–34‰.

Family: Polygordiade.

Genus: *Polygordius* Schneider, 1868.

Polygordius madrasensis Aiyar and Alikunhi, 1944.

Aiyar and Alikunhi (1944) described the species from the intertidal sands on the coasts of Madras and Krusadai Island. The local forms conform to the original description and reach a length of 5–7 mm. The worms were commonly encountered throughout the year in coarse and medium sands 5–10 cm. below surface between the low and the mid-water levels. The species is negatively phototactic and gregarious in habits.

Polygordius uroviridis Aiyar and Alikunhi, 1944

Aiyar and Alikunhi (1944) described the species from the beach sands at Madras. The forms were frequently encountered in coarse and medium sands between the low and the mid-water levels of the beach, in association with *P. madrasensis*. The worm is sluggish in habits and when disturbed coils into a mass adhering firmly to the substrate with the help of its adhesive pygidium.

Family: PROTODRILIDAE.

Genus: *Protodrilus* Hatschek, 1881.

Protodrilus pierantonii Aiyar and Alikunhi, 1944

Aiyar and Alikunhi (1944) described the species from the coasts of Madras, Gulf of Mannar and Cranganore. The local forms are identical with the Madras species in all its essential features and were commonly encountered throughout the year in coarse and medium sands 10 cm. below surface between the low and the mid-water levels. The species is quite active and gregarious in habits.

Protodrilus indicus Aiyar and Alikunhi, 1944

Aiyar and Alikunhi (1944) described the species from the intertidal zone at Madras. The forms were frequently collected in medium sands near the mid-water level in the company of *P. pierantonii*. The worm is negatively phototactic and sluggish in habits.

Family: SACCOCIRRIDAE.

Genus: *Saccocirrus* Bobretzky, 1872.

Saccocirrus minor Aiyar & Alikunhi, 1944

Aiyar and Alikunhi (1944) described the species from the beach sands at Madras and Cranganore. The Madras specimens attain a length of 10–15 mm. while the local forms are smaller reaching only 7–8 mm., the

species was rarely encountered in coarse sands with fine shell gravel 10 cm. below surface near the low water level. The species is quite active and carnivorous, feeding on the smaller microfauna of the sand.

Sacocirrus cirratus Aiyar and Alikunhi, 1944

Aiyar and Alikunhi (1944) described the species from the intertidal zone at Madras. The species was rarely encountered on this coast, occurring in coarse sands with fine shell gravel 15 cm. below surface near the low water level.

Family: NERILLIDAE

Genus: *Nerilla* Schmidt, 1848.

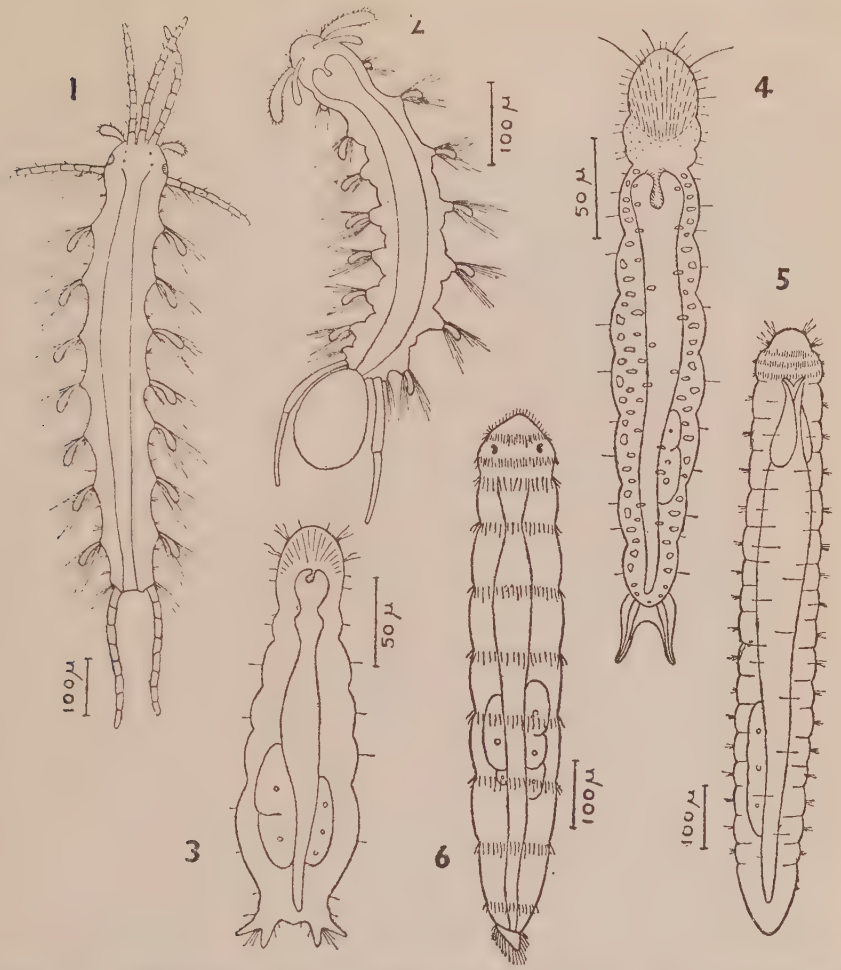
Nerilla antennata Schrnidt, 1863 (Fig. 1)

This species has a wide geographical distribution and its occurrence has been commonly reported in Europe on the coasts of Baltic, North Sea, North Atlantic and Mediterranean (*see* Fauvel, 1927). It is also known from Brazil (Marcus, 1947) and South-West Africa (Remane, 1949) on the Atlantic coast and Puget Sound (Wieser, 1957) on the Pacific Coast. The individuals on this coast agree with the type description of the species and attain a length of 0·8 mm. excluding the tentacles and anal cirri. Some geographical variations of the lateral ciliary tufts of the species has been reported (*see* Wieser, 1957). In the local forms the lateral cilia between parapodia consists of two tufts on each side. This species was rarely encountered on this coast in coarse and medium sands 10–15 cm. below surface between the low and the mid-water levels of the beach. The worms are transparent matching with the substrate and sluggish in habits.

Genus: *Nerillidium* Remane, 1925.

Nerillidium mediterraneum Remane, 1928 (Fig. 2)

Remane (1928) described the species from the beach sands at Naples. Later it has been reported on the Mediterranean coast at Naples (Gerlach, 1953; Boaden, 1965), Banyuls (Gerlach, 1954), and Marseilles (Swedmark, 1956) and the Atlantic coast of France (Renaud-Mornant and Jouin, 1965) and Africa (Remane, 1949). The local forms conform to the original description and reach a length of 0·5–0·6 mm. excluding the anal cirri. The palps and tentacles are more slender and longer than those of the Mediterranean forms. The anal cirri grow to a length of about 200 μ and are two-jointed resembling those of the species at Naples. Regeneration of the lost



FIGS. 1–6. Archiannelida. Fig. 1. *Nerilla antennata* Schmidt. Adult animal, dorsal view. Fig. 2. *Nerillidium mediterraneum* Remane. Adult animal, dorsal view. Fig. 3. *Diurodrilus minimus* Remane. Adult animal, dorsal view. Fig. 4. *D. benazzii* Gerlach. Adult animal, dorsal view. Fig. 5. *Trilobodrilus nipponicus* Uchida and Okuda. Adult animal, dorsal view. Fig. 6. *Dinophilus gyrociliatus* (Schmidt). Female, dorsal view.

cirri has also been observed. The parent carries a large solitary egg towed to the posterior border of the pygidium until it hatches and the young is released. The forms were commonly encountered throughout most of the year in medium sands 15 cm. below surface near the mid-water level. The species is sluggish and gregarious in habits.

Family: DINOPHILIDAE.

Genus: *Diurodrilus* Remane, 1925.

Diurodrilus minimus Remane, 1925 (Fig. 3)

Remane (1925) described the species from the beach sands at Kiel and Helgoland and later its occurrence has been reported from North Sea (Karling, 1954), Roscoff (Swedmark, 1955), Arcachon (Renaud-Debyser, 1963) and North Wales (Boaden, 1963). The local forms conform to the original description of the species and reach a length of 240 μ. Only a few specimens of this species were collected near half-tide level 20 cm. below surface in medium sands measuring 200–300 μ in their mean diameter. The archiannelid makes rapid gliding movements and was observed to browse about the substrate feeding on fine particles of detritus, bacteria and other smaller protozoans. The worm is thigmotactic and clings to sand particles when subjected to a current of water.

Diurodrilus benazzii Gerlach, 1952 (Fig. 4)

Gerlach (1952) described the species from the intertidal sands on the Italian coast. Later Delamare-Debouteville (1953) reported its occurrence from the Canet Beach on the French Mediterranean coast. The local specimens agree with the original description and attain a length of 300 μ. The forms were commonly encountered throughout the year in sands 15 cm. below surface near the half-tide level. The worms showed preference for substrates with medium sand grades measuring between 200–500 μ in their mean diameter. The animal's body is well adapted for life in the habitat as evident by the ease with which it moved in the interstices of the sand particles. The species is quite active and often lurks amidst sand grains. The worm makes rapid jerky movements or firmly sticks to the substrate with the help of its caudal adhesive forks whenever there is a commotion in the habitat due to wave action, etc. Its feeding habits are similar to those of *D. minimus*. The worm is negatively phototactic and gregarious in habits.

Genus: *Trilobodrilus* Remane, 1925.

Trilobodrilus nipponicus Uchida and Okuda, 1943 (Fig. 5)

Uchida and Okuda (1943) described the species from the coast of Japan. Wieser (1957) reported its occurrence in the beach sands at Puget Sound on the Pacific coast. The local forms are quite transparent and reach a length of 0·7–0·8 mm. A stiff spine characteristic of the species is present on the buccal segment and each trunk zonite bears laterally a pair of ciliary tufts. The worms were frequently encountered through most of the year in medium sands 15 cm. below surface near the mid-water level. The archiannelid is negatively phototactic and gregarious in habits. It is highly thigmotactic

and even the slightest commotion in the habitat, makes the worm contract and adhere firmly to the substrate.

Genus: *Dinophilus* Schmidt, 1848.

Dinophilus gyrociliatus (Schmidt) (Fig. 6)

This species has a wide geographical distribution in Europe occurring on the coasts of Atlantic and Mediterranean (*see* Fauvel, 1927). The local forms are transparent and attain a length of 0·7–0·8 mm. with conspicuous ciliary girdles on the trunk. Sexual dimorphism is present, the males being much reduced in size. The species are rare on this coast and only a few were collected in medium sands 20 cm. below surface between the low and the mid-water levels of the beach.

Summary

The present paper reports the occurrence of twelve species of archiannelids in the beach sands of Waltair coast, six of the species being new records for the Indian waters.

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SOME INTERESTING FUNGI

II. *Cercospora hygrophilae* Sp. Nov. and *Stenella plectroniae* Sp. Nov.

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Cercospora hygrophilae found on living leaves of *Hygrophila spinosa* and *Stenella plectroniae* found on *Plectronia parviflora* have been described and illustrated.

23. *Cercospora hygrophilae* Ponnappa sp. nov. (Fig. 1)

Infection patches amphigenous, mostly along the margin of the leaf blade, few, oblong, oval or irregular, dark brown to almost black, measuring 5–15 mm. in diameter. Fruiting amphigenous. Stroma composed of few globular to irregular brown, thick-walled cells. Conidiophores medium-olivaceous to brown, single or in fascicles of 5–15, simple, straight or bent, 1–3 septate, geniculate, with a terminal conspicuous thickened truncate conidial scar, 28·0–41·0 × 2·75–4·0 (33·1 × 3·3 average) μ. Conidia hyaline, straight or curved, obclavate to acicular indistinctly multiseptate (5–15) measuring 52·0–171·0 × 1·25–2·0 (122·5 × 1·6 average) μ.

On living leaves of *Hygrophila spinosa* T. Anders. February 21, 1967. Bannerghatta (Mysore State). K. M. Ponnappa, Herb. I.M.I. 126157 (type).

Cercospora hygrophilae Ponnappa sp. nov. (Fig. 1)

Maculae paucae, amphigenae, praecipue marginem folii insidentes, oblongue, ovales vel irregulares, dense brunneae fere atrae, 5–15 mm. latae. Caespituli amphigeni. Storma excellulis paucis globularibus parietibus crassis constans. Conidiophora modice olivacea, singularia vel 5–15 in fasciculo oriunda, simplicia, recta vel curvata, geniculata, 1–3 septata, 28·0–41·0 × 2·75–4·0 μ. Conidia hyalina, recta vel curvata, obclavata vel acicularia, indistincte 5–15 septata, 52·0–171·0 × 1·25–2·0 μ.

FIG. 1. *Cercospora hygrophilae.* (A) Habitat, (B) Conidiophores and (C) Conidia.

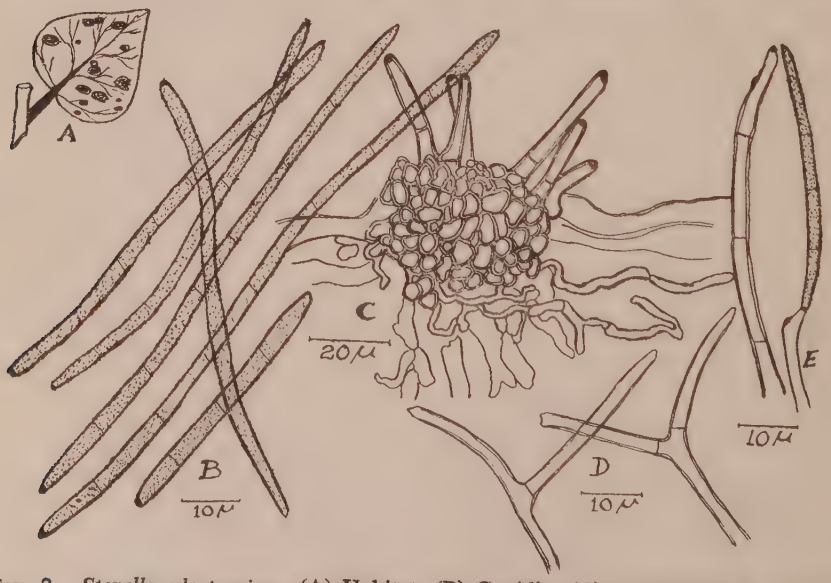

FIG. 2. *Stenella plectroniae.* (A) Habitat, (B) Conidia, (C) Conidiophores, (D) Branches conidiophores, (E) Conidiophore with conidia attached.

Habitat in foliis vivis *Hygrophilae spinosae* T. Anders. Bannerghatta, Mysore State, India, 21–II–1967, leg., K. M. Ponnappa, Herb. IMI 126157, typus.

24. *Stenella plectroniae* Ponnappa sp. nov. (Fig. 2)

Spots amphigenous, few to many, circular to oval with a dark brown centre surrounded by light brown dead tissue of the host, beyond which there is a prominent brown margin with light yellow periphery measuring 1–5 mm. in diameter. Adjacent spots coalesce to form large patches of 10–12 mm. of irregular shape. Fruiting amphigenous, stroma composed of globular to rectangular dark brown cells and measure on an average 41·75 μ. Mycelium septate, branched, hyaline and the secondary superficial mycelial hyphae are finely rough-walled, 1·75–2·75 μ wide. Conidiophores smooth, simple or branched, in fasciciles or arising as branches from superficial repent rough-walled secondary mycelium, cylindrical, straight or bent, brown to light brown, thick-walled, 1–5 septate, geniculate 24·0–74·50 × 2·75–3·0 (51·25 × 3·0 average) μ, conidial scar conspicuous measuring 1·75–2·0 μ. Conidia subhyaline to light brown, obclavate to cylindrical, straight or bent, distinctly rough-walled, catenulate, showing conspicuous hila, 3–15 septate, measuring 46·50–173·0 × 1·75–2·75 (101·50 × 2·25 average) μ, and the apices of the terminal conidia not showing the scar are obtuse.

On living leaves of *Plectronia parviflora* Bedd. March 2, 1967, Agara, Bangalore (Mysore State). K. M. Ponnappa, Herb. IMI 126158 (type).

Stenella plectroniae Ponnappa sp. nov. (Fig. 2)

Maculae amphigenae, paucae vel numerosae, orbiculares vel ovales, pallide brunneae centro dense brunneae, margine brunneo distincto, 1–5 mm. latae, zona pallide flavida cinctae, saepe coalescentes. Caespituli amphigeni. Stroma circa 42 μ latum, excellulis dense brunneis globularibus vel rectangularibus constans. Mycelium primarium immersum, exhyphis septatis, ramosis, hyalinis compositum. Mycelium secundarium superficiale, hyphis repentibus, minute aspris, 1·75–2·75 μ latis. Conidiophora modice vel pallide brunnea, fasciculata vel exmycelio secundario singulatim oriunda, cylindrica, simplicia, laevia, recta vel curvata, parietibus crassis, 1–5 septata, geniculata, 24·0–74·50 × 2·75–3·75 μ. Cicatrices conidiales conspicuae, incrassatae. Conidia subhyalina vel pallide brunnea, obclavata vel cylindrica, recta vel curvula, parietibus distincte aspris, catenulata, hilis conspicuis incrassatis, 3–15 septata, 46·50–173·0 × 1·75–2·75 μ.

Habitat in foliis vivis *Plectroniae parviflorae* Bedd., 2–III–1967, Agara, Bangalore, Mysore State, India, Leg. K.M. Ponnappa, Herb. IMI 126158, typus.

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